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Electrostatic parameters of cationic liposomes commonly used for gene delivery as determined by 4-heptadecyl-7-hydroxycoumarin.

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Cationic liposomes are used to deliver genes into cells in vitro and in vivo. The present study is aimed to characterize the electrostatic parameters of cationic, large unilamellar vesicles, 110 +/- 20 nm in size, composed of DOTAP/DOPE (mole ratio 1/1), DOTAP/DOPC (mole ratio 1/1), 100% DOTAP, DMRIE/DOPE 1/1, or DC-CHOL/DOPE (mole ratio 1/1). {Abbreviations: DOTAP, N-(1-(2,3-dioleoyloxy)propyl)-N,N-trimethylammonium chloride; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine; DMRIE, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethylammonium bromide; DC-CHOL, 3beta[N-(N',N'-dimethylaminoethane)carbonyl]cholesterol}. The cationic liposomes had a large positive surface potential and a high pH at the liposomal surface in 20 mM Hepes buffer (pH 7.4) as monitored by the pH-sensitive fluorophore 4-heptadecyl-7-hydroxycoumarin. In contrast to DOTAP and DMRIE which were 100% charged, DC-CHOL in DC-CHOL/DOPE (1/1) liposomes was only about 50% charged in 20 mM Hepes buffer (pH 7.4). This might result in an easier dissociation of bilayers containing DC-CHOL from the plasmid DNA (which is necessary to enable transcription), in a decrease of the charge on the external surfaces of the liposomes or DNA-lipid complexes, and in an increase in release of the DNA-lipid complex into the cytosol from the endosomes. Other electrostatic characteristics found were that the primary amine group of DOPE in cationic liposomes dissociated at high (> 7.9) pHbulk and that a salt bridge was likely between the quaternary amine of DOTAP or DMRIE and the phosphate group of DOPE or DOPC, but not between the tertiary amine of DC-CHOL and the phosphate group of DOPE. The liposomes containing DOTAP were unstable upon dilution, probably due to the high critical aggregation concentration of DOTAP, 7 X 10⁻⁵ M. This might also be a mechanism of the dissociation of bilayers containing DOTAP from the plasmid DNA.

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